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Investigating the Effects of Natural Environmental Factors on the Viability of Dye Tracer Testing

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INVESTIGATING THE EFFECTS OF NATURAL ENVIRONMENTAL FACTORS ON THE VIABILITY OF DYE TRACER TESTING

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INTRODUCTION

Roughly 18 percent of the United States is underlain by karst rock or geologic features that have the potential to develop into karst (Weary and Doctor, 2014), which is defined by its soluble nature and ability to hold large amounts of water. About 20-25% of the world's population relies on karst aquifers to provide clean water for drinking, irrigation, or industrial use (Ford and Williams, 2013). Karst most commonly forms within rocks comprised of soluble minerals, including carbonate rocks. Carbonates have the potential to dissolve as slightly acidic waters pass through the cracks and crevices in the rock. Over time, cracks and crevices can widen to form more efficient conduits and sometimes extensive cave systems that store and transmit significant volumes of groundwater.

One of the most productive karst aquifers in the United States is the Edwards Aquifer located in central Texas (Smith et al., 2005). The Edwards Aquifer is made of faulted Cretaceous limestone and features caves, sinkholes, and artesian springs. The Edwards Aquifer covers about 4,350 square miles and provides water for over 1.7 million people. The population of people depending on the Edwards Aquifer will continue to grow as San Antonio, which relies on the Edwards Aquifer for drinking water, is one of the fastest growing cities in America (Smith et al., 2005). Unfortunately, karst aquifers, like the Edwards, are vulnerable to contamination due to their poor filtration and easily accessible recharge points (Kaçaroğlu, 1999). This vulnerability puts those reliant on water from the aquifer, including cave-dwelling endangered species, at risk of exposure to contaminants (Shockey, 1996).

Because the Edwards Aquifer is such a critical water resource, the Edwards Aquifer Authority and similar water protection agencies have studied the hydrogeologic characteristics of the aquifer extensively. For example, Hauwert et al. (2004) exercised seven different methodologies in order to better understand local flow systems. Others have conducted field-

based experiments to observe localized flow paths (Taucer et al., 2005). Despite this research, past groundwater flow modeling has consistently underestimated flow velocity and mischaracterized flow pathways in the Edwards Aquifer (Smith et al., 2005). This mischaracterization is due, in part, to the fact that water does not flow through the rock matrix of karstic aquifers, but instead flows through a less-predictable network of conduits and caverns (Smith et al., 2005).

In order to avoid the errors produced by inaccurate modeling, researchers use dye tracer testing to understand groundwater flow in the Edwards Aquifer (Hauwert et al., 2004; Hunt et al., 2005; Smith et al., 2005; Taucer et al., 2005). Dye tracer tests involve releasing fluorescent dye (uranine, eosine, rhodamine variants) into an aquifer via streams or sinkholes. Researchers place granular activated carbon (GAC) along the suspected flow path in order to passively detect dyes in water flowing through wells, springs, and streams. As the fluorescent dyes pass the GAC, the dye molecules adsorb to the GAC. Once a test is complete, the GAC packet is collected and analyzed for dye. If dye is extracted from the packet, then the researcher can conclude that the injection point is connected to the GAC packet's placement point by a flow path (Smith et al., 2005).

Dye tracer testing is a viable method due to GAC's capacity to electrostatically interact with molecules around it (Smart and Simpson, 2002). GAC is a complex molecule with many different functional groups (carboxyl, hydroxyl, phenolic, etc.) that have the ability to interact with other molecules in solution. Similarly, dye molecules also have functional groups that can interact with other molecules. As dye molecule pass by the GAC, the functional groups on the dye and GAC can interact electrostatically and thus the dye is adsorbed and removed from the aqueous phase. The electrostatic interaction holds the dye to the GAC until the GAC is placed in

a basic solution that releases the dye back into the aqueous phase.

Unfortunately, fluorescent dyes can be affected by water chemistry or other environmental variables that can affect the adsorption of dye to GAC (Smart and Laidlaw, 1977). For example, some dyes are sensitive to light and/or temperature and will chemically decay when exposed to sunlight and heat, thus rendering them incapable of adsorption (Smart and Laidlaw, 1977). Previous work has shown that pH can affect the fluorescence of some dyes, even in the natural range of pH values (Flury and Wai, 2003). Water quality factors, such as chloride content, can also effect dyes by altering their fluorescence (Smart and Laidlaw, 1977).

Dye may also face competitive adsorption with other molecules in the aqueous phase. Contaminants in water may interact with GAC and hinder the dye's ability to interact with the GAC. One common contaminant in the field is tannic acid (Rivera-Utrilla et al., 1993). Tannic acid is naturally occurring and is released into the environment as plant life decays. There are three mechanisms by which tannic acid could inhibit dye adsorption: 1) occupying adsorption sites, thus inhibiting dye adsorption, 2) desorbing dye that was previously adsorbed to GAC, or 3) forming an aqueous complex that would inhibit the adsorption of dye to GAC. The combined effects of these environmental factors make the adsorption of fluorescent dye to GAC complex.

In order to assist the Edwards Aquifer Authority in understanding the complexities of dye tracer testing, the aim of this research is to investigate how uranine dye and GAC interact with each other under various controlled conditions. We investigate the kinetic properties of dye adsorption, determine whether or not naturally-occurring tannic acid affects the sorptive capacity of dye onto GAC, and measure the impact of pH upon dye adsorption onto GAC. Our results provide insight into the competitive adsorption that fluorescent dyes may experience in natural aquifer systems, thus putting quantitative constraints on processes that could inhibit dye

adsorption and lead to false negatives in dye tracer tests. This study will help researchers working in karst environments understand some of the shortcomings of dye tracer testing in uncontrolled field environments.

METHODS

Solution Preparation

Uranine solutions were prepared using Nanopure water (18.0 mΩ-cm) and Chromatech Chromatint® Uranine Liquid Concentrate (stock powder concentration = 73.85% dye). Concentrations of prepared uranine solutions ranged from 0.01 ppb to over 300 ppm depending on the intended method of analysis. Final dye concentrations were made by serial dilution to minimize error.

Tannic acid solutions were prepared using Nanopure water (18.0 mΩ-cm) and reagent grade tannic acid (Sigma Aldrich; 99.5% purity). 10 ppm tannic acid solutions were prepared via serial dilution.

Base solutions used to extract the dye from the GAC were created using potassium hydroxide (KOH). Extractant solutions were made by dissolving solid KOH with 70% isopropyl alcohol to create a solution that is 95% isopropyl alcohol solution and 5% KOH.

GAC Preparation

Granular activated carbon (GAC), supplied by the Edwards Aquifer Authority, was sieved to sizes between 1.18 mm and 2.00 mm. Sieved GAC was then washed with Nanopure water until the water ran clear through the GAC to remove fine particulates. The washed GAC was laid out in a thin layer to dry at room temperature for 12 hours.

Batch Reactor Experiments

Batch reactor experiments using uranine concentrations of 10, 25, 50, 90, 150, 185, 200, 250, 300, and 369 ppm were sampled at 2.5 hours and 5 hours in order to understand how different concentrations of dye adsorb to GAC over time. Two grams of GAC were placed in a mesh packet in 40 mL of solution that was stirred at a constant rate throughout the experiment. Samples taken from the batch reactor did not exceed 0.5 mL to keep the volume in the reactor relatively equal throughout. Dye solution in the batch reactor was analyzed using a UV-Visible GENESYS 150 Spectrophotometer at 490 nm.

In order to determine the equilibration time needed for the dye to adsorb to the GAC, batch reactor experiments were conducted using 30 ppm solutions. Twenty-five grams of GAC were placed in 500 mL of solution, and the dye solution in the batch reactor was sampled every minute for the first five minutes, every ten minutes for the next hour, and every thirty minutes until the dye was no longer detectable. Samples taken from the batch reactor did not exceed 0.5 mL in order to keep the volume in the reactor relatively equal throughout. Samples were analyzed by the UV-Visible GENESYS 150 Spectrophotometer at 490 nm.

Batch reactor experiments were conducted in order to understand the relationship between the concentration of dye in water and dye extracted from GAC and analyzed by a Perkin Elmer LS-50B luminescence spectrometer. Approximately 25 g of rinsed GAC (1.18-2.00 mm) was placed into permeable packets of mesh screen purchased from the hardware store. One GAC packet was put into a 1000 mL beaker and secured with a stir rod and tape to ensure that the packet was suspended in the water column. The GAC was suspended in 500 mL of a standard uranine solution (20 ppb, 10 ppb, 5 ppb, or 2.5 ppb), which was stirred at a constant rate for five hours. After five hours, the 25 g GAC packet was air dried overnight (12 hr). Dyes were

extracted from the GAC by leaving the GAC for one hour in a 95% isopropyl alcohol solution and 5% KOH solution. The extract was then stored in glass cuvettes until it was analyzed using the Perkin Elmer LS50B luminescence spectrometer. An outline of the procedure is included in Figure 1.

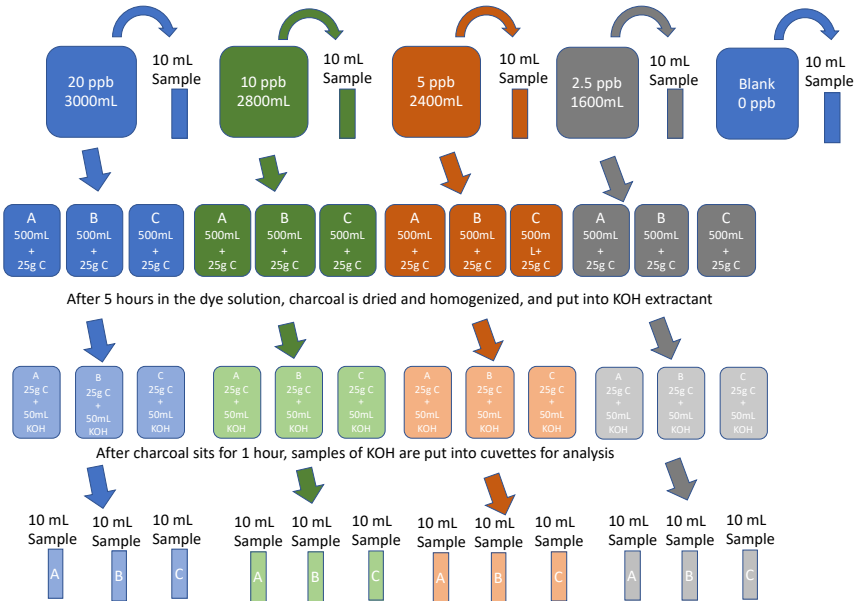


Figure 1. Procedure to test the relationship between dye concentration and adsorption to GAC

Mixed Flow Reactor Experiments

In order to test the impact that tannic acid may have on dye adsorption to GAC, two grams of sieved GAC were placed on top of the grating inside the 3D-printed mixed flow reactor perpendicular to the direction of water flow (Michel et al. 2018). Experiments were conducted either with 20 ppm or 500 ppb of uranine dye, concentrations chosen due to the results found during the batch reactor experiments. MFR experiments proceeded using one of two methods. In the first method, 60 mL of 10 ppm tannic acid was injected into the mixed flow reactor using a syringe pump (Harvard Apparatus Standard Infuse/Withdraw PHD ULTRA) with a constant injection rate of 0.5 mL/min for two hours. After two hours, 60 mL of uranine dye solution

(either 20 ppm or 500 ppb) was injected into the mixed flow reactor using the same injection procedure as the tannic acid (Figure 2). A control test using Nanopure in place of tannic acid was conducted simultaneously in a second MFR. After the solutions were flushed though the mixed flow reactor, the GAC was removed and air-dried overnight (12 hrs). In the second method, uranine dye solution was injected prior to the injection of tannic acid solution, otherwise following the same experimental design. Dye was extracted from the GAC by immersing it in extractant solution for one hour. The extractant solution was analyzed using the UV-Visible GENESYS 15 spectrophotometer.

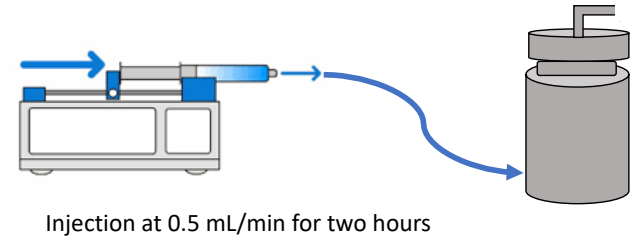


Figure 2. Mixed flow reactor and syringe pump set up and procedure. Solutions of uranine dye, tannic acid, or Nanopure water (for control) are injected into the MFR at 0.5 mL/min for two hours using the syringe pump.

In order to understand the effect of pH on dye's adsorption to GAC, MFR experiments were conducted using pH-adjusted uranine solutions ranging from 0.01- 5 ppm. Two grams of GAC was placed in uranine solution that was then adjusted for pH using either 0.5 M hydrochloric acid (HCl or 0.5 M sodium hydroxide (NaOH). Experiments were conducted at pH 4.5 or pH 8.5 because these values are near the pKa values of uranine dye where functional groups undergo (de)protonation reactions that either generate or neutralize negative surface charge. The uranine solution was injected into the mixed flow reactor using a syringe pump with a constant injection rate of 0.5 mL/min for two hours. The GAC was removed after injection and air-dried overnight (12 hrs). A control test was conducted with dye in Nanopure water that was

not pH adjusted for comparison. Dye was extracted from the GAC by immersing it in extractant solution for one hour. The extractant solution was then analyzed using the Perkin Elmer LS-50B luminescence spectrometer.

Mixed Flow Reactor Design

3-D printed mixed flow reactors (MFRs) were used to simulate a natural flowing environment in a laboratory setting. They give the researcher control over experimental characteristics such as flow rate, solute chemistry and concentration, mass/volume ratios for sorbent and sorbate, pH, reactor content, and other variables (Michel et al., 2018). MFRs are ideal for low temperature geochemical experiments because they are durable at high and low pH as well as temperatures up to 80°C (Kletetschka et al., 2018). The reactors were manufactured using inverse stereolithography desktop 3D printing where liquid photopolymer resin was hardened layer by layer using a laser beam (Figure 3). This method produces models with less permeability, porosity, and roughness than traditional 3D printing methods. MFRs were printed using a Formlabs Form 2 printer with a layer thickness of 50 μm (Michel et al, 2018). Reactors feature two inlets, space for a 12.7 mm stir bar, a grated platform, a 25 mL internal chamber, and one outlet (Figure 4).

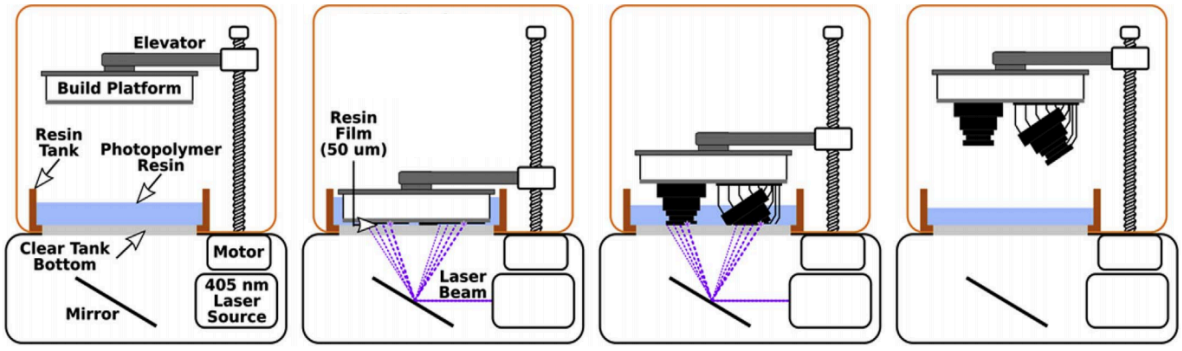


Figure 3. Inverse stereolithography 3D printing process. The black shapes visible in the diagrams on the right are the forms produced by the printing process. Modified from Michel et al. (2018).

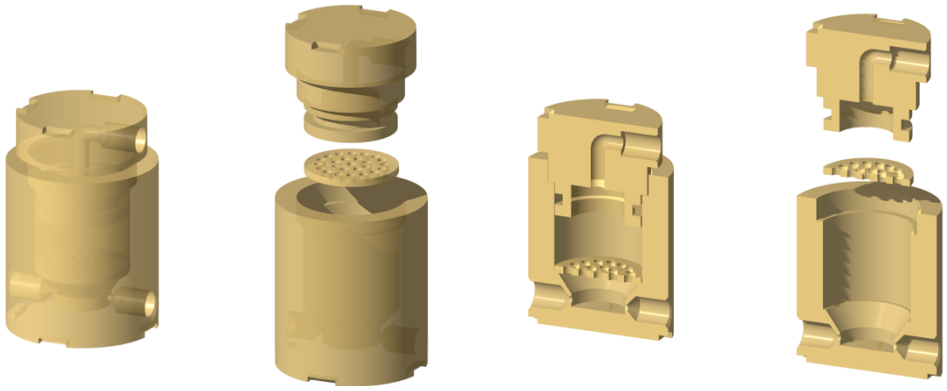


Figure 4. Model of 3D printed mixed flow reactor showing components and cross-sectional views. This model has two inlets at the bottom, a single outlet at the top, and a removable grate. Images courtesy of Aly Hoeher.

RESULTS

Range of detectable dye adsorption on GAC

To evaluate the detectable ranges of uranine concentrations for our analytical instruments, I performed a preliminary screening of instrumental response to a range of concentrations. Samples analyzed with the UV-Visible GENESYS 150 spectrophotometer were detectable between 500 ppb and 20 ppm because a discernable absorbance spectrum, with absorbance maxima at ~504 nm, was observed to increase approximately linearly within this concentration range (Figure 5). At higher concentrations, absorbance spectra become non-linear and maxima plateau around absorbance values of ~2.5. The Perkin Elmer LS-50B luminescence spectrometer was substantially more sensitive, producing a clear luminescence peak at 504 nm for extractant uranine concentrations as low as 2.5 ppb (Figure 6). Based on these results, we limited the initial uranine concentration for experiments using UV-Vis spectrophotometry to between 500 ppb and 20 ppm. For experiments at lower concentrations (between 2.5 and 20 ppb), we used the luminescence spectrometer.

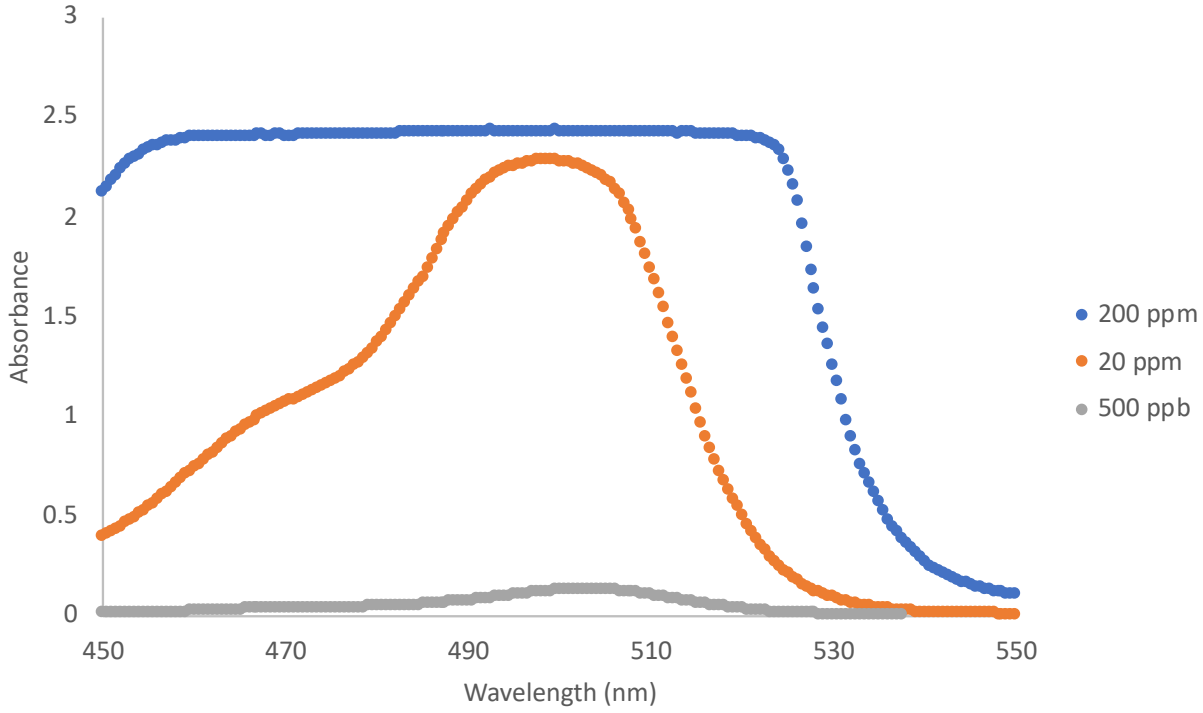


Figure 5. UV- Visible spectral response from 450-550 nm for three uranine solutions in Nanopure matrix.

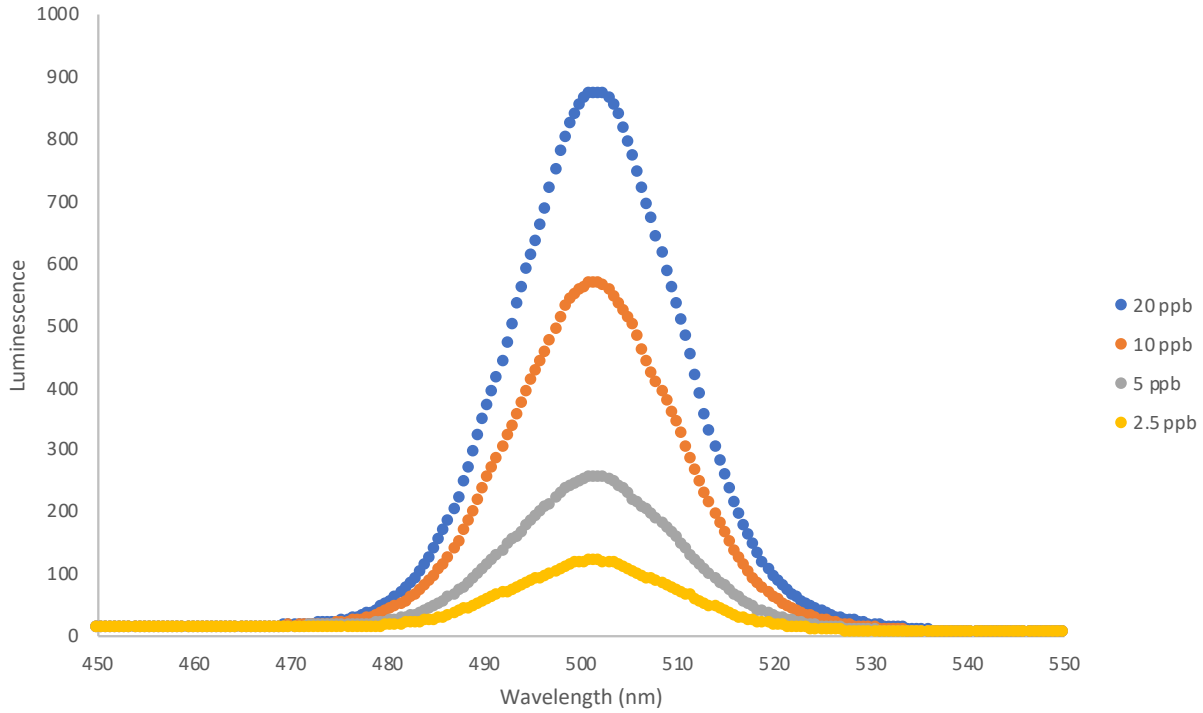


Figure 6. Luminescence spectrometer response from 450-550 nm for four uranine solutions in KOH matrix.

To account for matrix effects that might cause analytical disparities between the injection solution matrix (Nanopure water) and the extraction solution matrix, we also analyzed standard solutions of uranine in the extraction solution matrix (95%/5% KOH/isopropanol) with the UV-Visible GENESYS 150 spectrophotometer. A regression of the absorbance maxima (spectra not shown; absorbance maxima at 501 nm) resulted in a linear relationship for concentrations from ~16 ppb to 20 ppm (Figure 7). However, a notable shift to higher absorbance values was observed in the KOH matrix (i.e., 20 ppm in the water matrix = 1.3 absorbance units vs. 20 ppm in KOH matrix = 2.3 absorbance units). This regression curve was used to calculate concentrations of uranine in subsequent chemical extraction experiments.

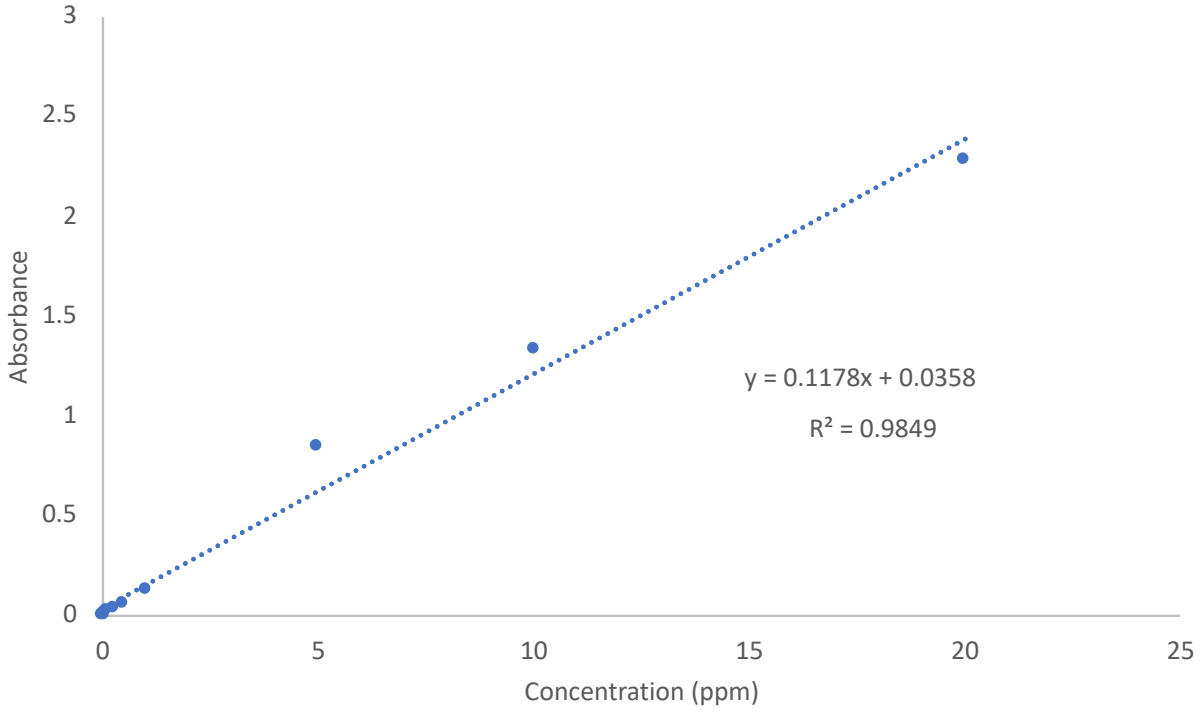


Figure 7. Regression curve showing the maximum absorbance of multiple dye concentrations in isopropanol/KOH solutions analyzed using the UV-Visible GENESYS 150 spectrophotometer.

Determining equilibration time

In order to understand how concentration affects the time it takes for dye and GAC to reach equilibrium, batch reactor experiments with uranine concentrations ranging from 10 ppm to over 360 ppm were monitored over a span of 5 hours. The water samples taken from the batch reactors and analyzed with the UV-Visible GENESYS 150 spectrophotometer at 490 nm show that concentrations at or below 100 ppm are approximately 99% adsorbed to the 2 g of GAC after 2.5 hours (Figure 8). After 5 hours, concentrations nearly at or below 200 ppm are approximately 97% percent absorbed to 2 g of GAC (Figure 8). Based on these experiments, we assume that experiments conducted below 100 ppm should reach equilibrium during a two-hour run time.

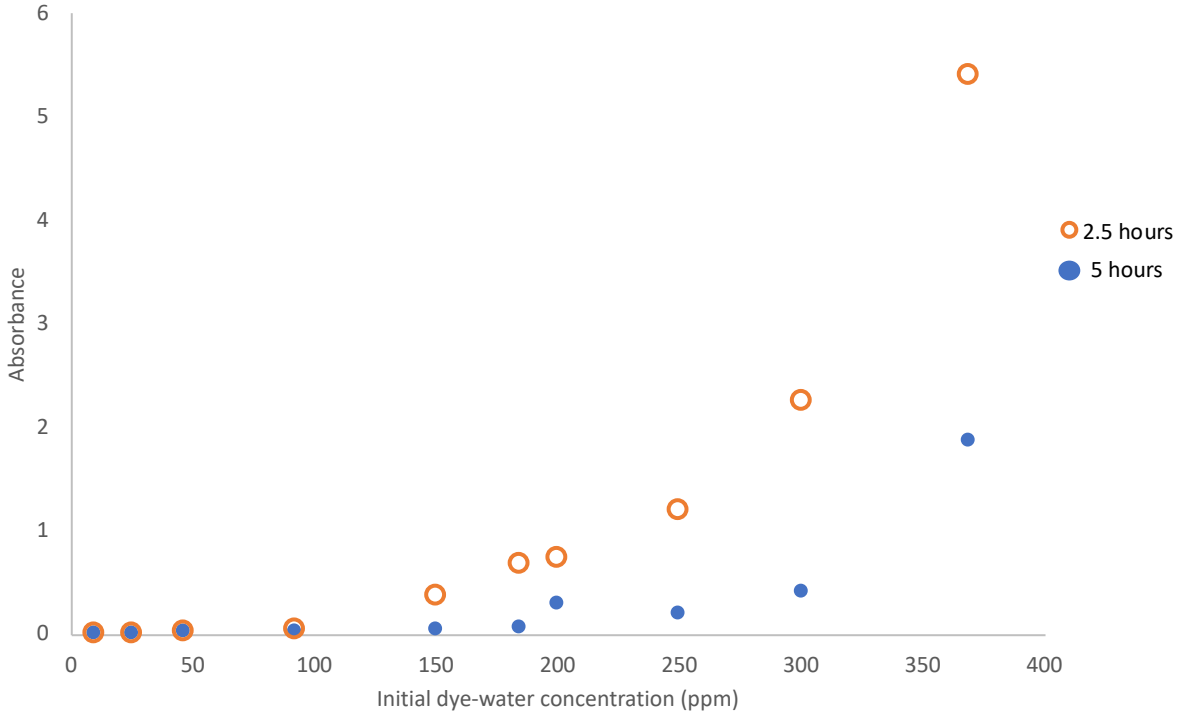


Figure 8. Absorbance response of water samples taken from batch reactors with set initial concentrations of dye in water. The blue data points represent water samples take after 2.5 hours of interaction with GAC. The orange data points represent water samples taken after 5 hours of interaction with GAC.

To closely evaluate the adsorption rate and equilibrium time of a single concentration of uranine and GAC, we performed a batch reactor experiment with 30 ppm uranine in water and

monitored the reactor until the dye was no longer detectable. Water samples taken from the batch reactor and analyzed with the UV-Visible GENESYS 150 spectrophotometer at 490 nm show that the dye was undetectable at 125 minutes (Figure 9). In fact, within the first 35 minutes, about 70% of the dye had already adsorbed to the GAC. After 2.5 hours, the 4 mg of dye initially injected into the solution were almost completely adsorbed by the 2 g of GAC.

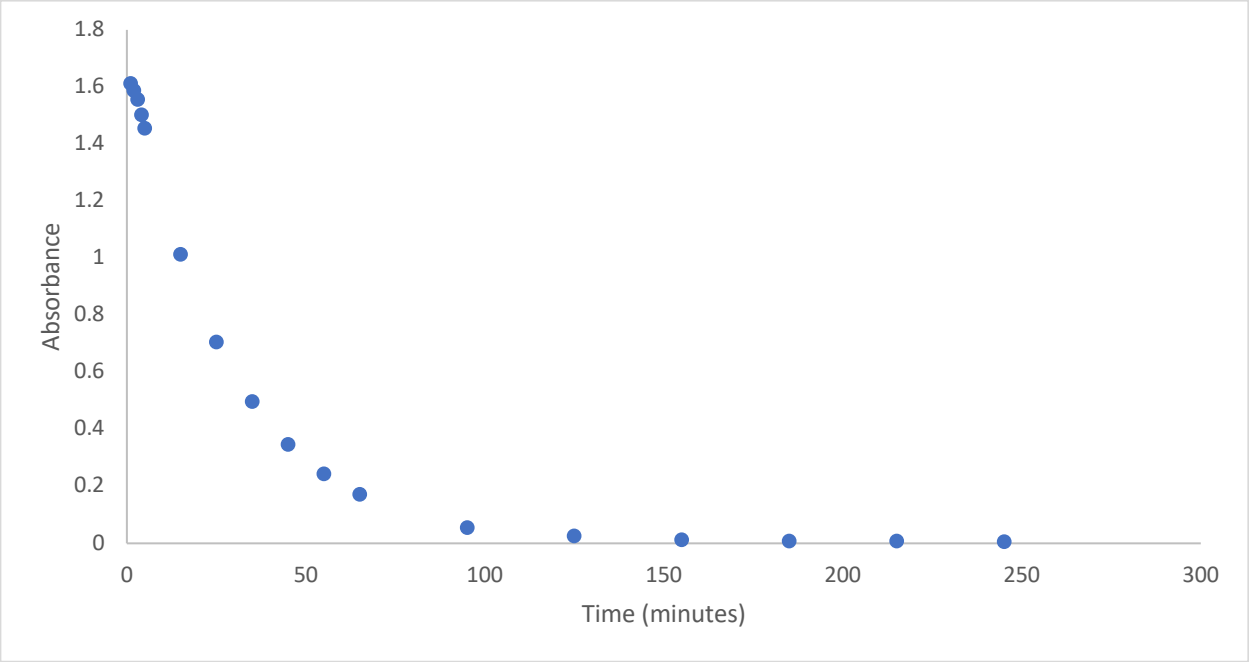


Figure 9. Time vs absorbance response of 30 ppm uranine dye in water showing interaction of dye and GAC.

Dye adsorption on GAC during batch reactor experiments

Batch reactor experiments at initial concentrations of 2.5, 5, 10, and 20 ppm uranine were performed to understand the relationship between concentration and luminescence. These experiments, conducted using the Perkin Elmer LS-50B luminescence spectrometer, reveal a quasi-linear relationship between initial concentration and luminescence value (Figure 10). The best-fit line calculated with these data has an R^2 value of 0.8567. These results show that there is a near direct relationship between the amount of dye that is initially placed in water and the

amount of dye that is extracted from GAC. However, these data show a substantial amount of variation between replicated experiments.

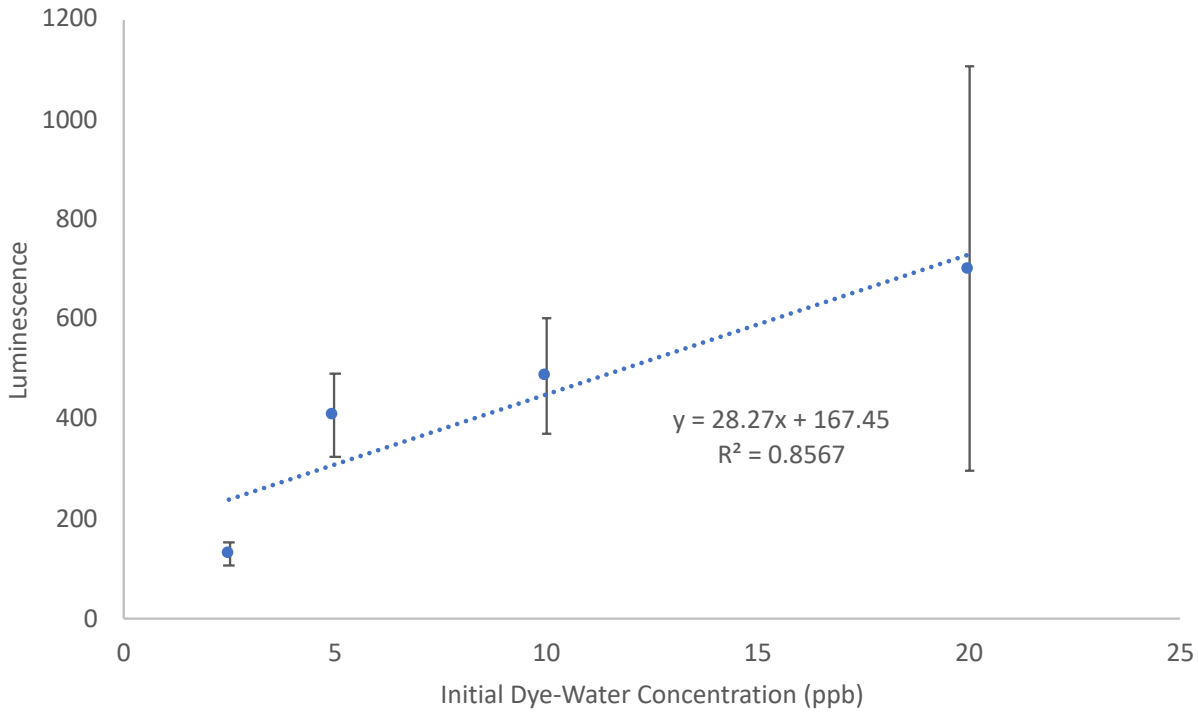


Figure 10. Concentration vs luminescence with initial concentration of dye in water plotted on the x-axis and the luminescence response of dye in KOH extracted from GAC. Error bars represent +/- one standard deviation from the average of the experimental replicates' maximum luminescence peak.

Competitive adsorption with tannic acid

20 ppm uranine experiments

Experiments to characterize competitive adsorption between 20 ppm uranine and tannic acid were conducted with mixed flow reactors using one of two methods. The first method consisted of injecting 60 mL of either 10 ppm tannic acid solution or DI water (as a control) into the mixed flow reactor followed by an injection of 60 mL of the 20 ppm uranine dye solution (1.2 mg of dye). This method was designed to evaluate if tannic acid can inhibit the adsorption of dye by occupying adsorption sites on the GAC, thus leaving no (or fewer) adsorption sites available for uranine. This injection procedure was used for four identical experiments that

yielded a wide range in extracted dye masses (Figure 11). For example, both the control and tannic acid-treated samples in experiment three yielded ~7 times more dye than experiment one. Additionally, the yield of the tannic acid-treated sample compared to the control varied widely across the four experiments. In experiment one, the control sample yielded 14.06% less dye than the tannic acid treated, and in experiment two, the tannic acid treated sample yielded 46.65% less dye than the control sample. Experiment three yielded similar, large yields for both the tannic acid sample and the control sample, and experiment four yielded a control sample mass 6.36% less than the tannic acid treaded sample.

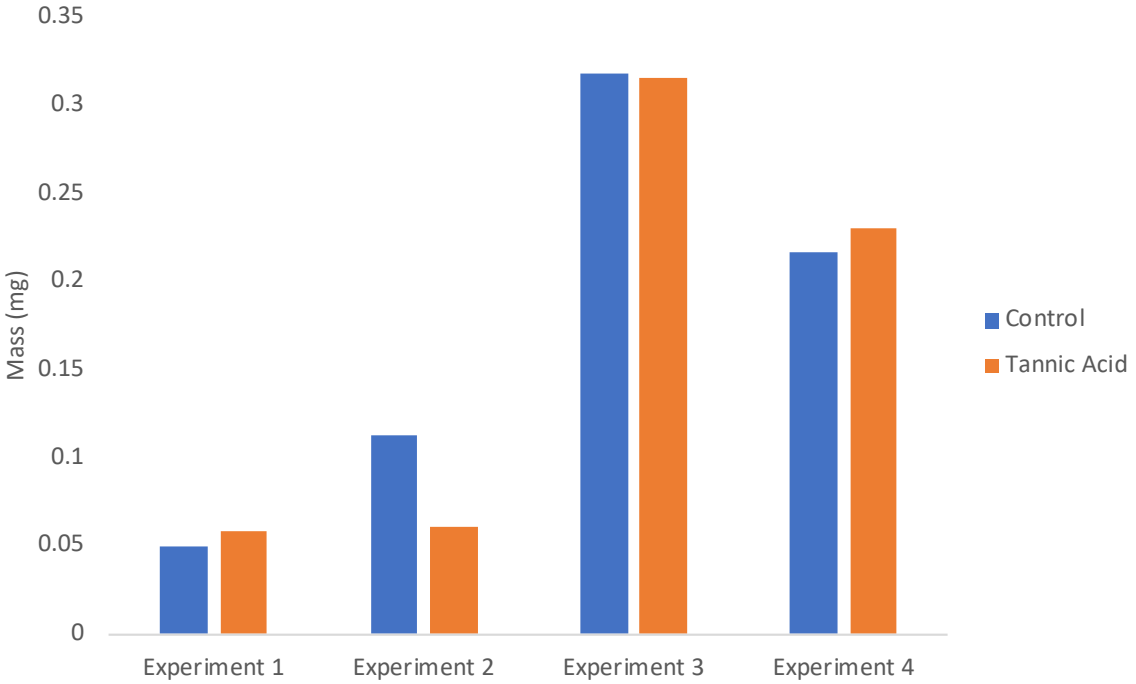


Figure 11. Bar graph of the mass of dye extracted from GAC in four MFR experiments where one sample was injected with tannic acid solution prior to 20 ppm uranine solution and the other acted as a control.

The second method consisted of injecting 60 mL of the 20 ppm uranine dye solution (1.2 mg of dye) into the mixed flow reactor followed by an injection of 60 mL of either 10 ppm tannic acid or 60 mL of Nanopure water as a control. This method aimed to deduce whether or not the tannic acid solution could desorb uranine off of the GAC after uranine had already passed

through the reactor and adsorbed to the GAC surface. Two identical experiments were conducted using this method. After the KOH/isopropanol extraction of GAC, experiment one (Figure 12) resulted in the control sample yielding 0.070 mg of dye in the extractant solution, and the tannic acid-treated sample yielded 0.069 mg, a small difference of 1.25%. Experiment two (Figure 12) resulted in the control sample yielding 0.057 mg of dye in the extractant while the tannic acid contaminated sample yielded 0.067 mg of dye in the extractant. No samples from these experiments yielded the entire 1.2 mg of dye that was originally introduced into the system.

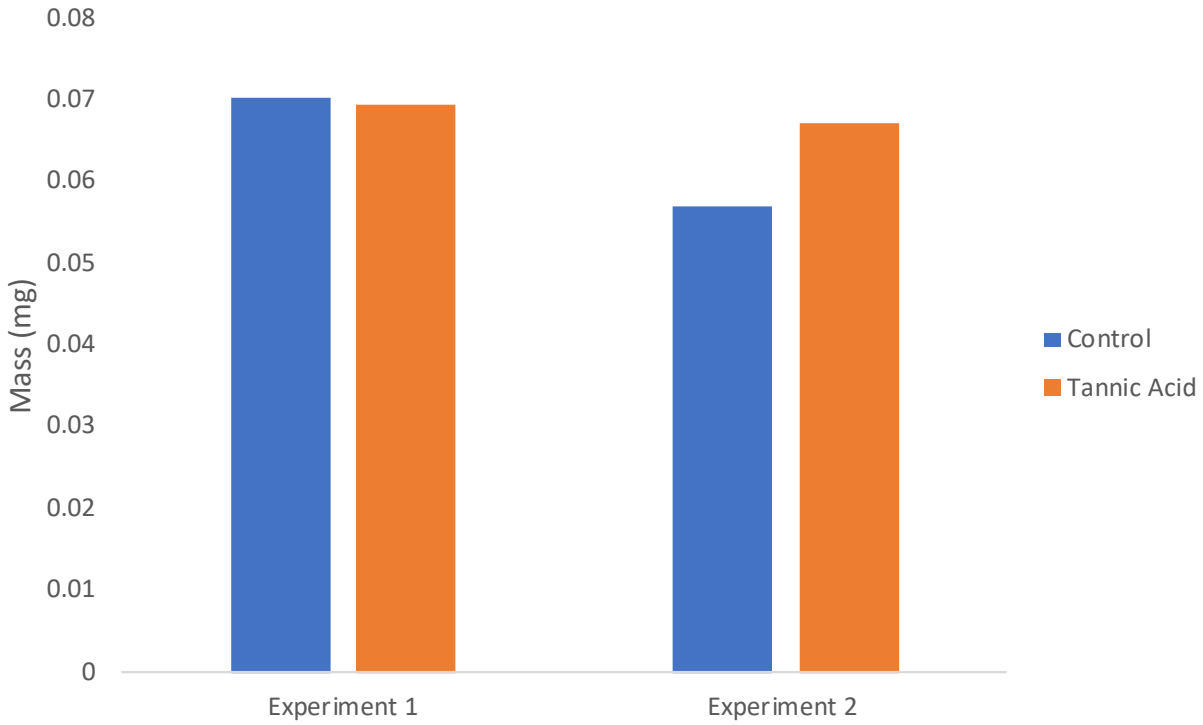


Figure 12. Mass of dye extracted from GAC in two MFR experiments where one sample was injected with 20 ppm uranine solution prior to tannic acid solution (orange) and the other acted as a control using Nanopure (blue).

500 ppb Uranine experiments

Experiments with 500 ppb of uranine were conducted in one of two ways, similar to the 20 ppm experiments. The first set of four identical experiments was conducted by injecting 60 mL of tannic acid prior to injection of 500 ppb uranine solution in order to determine if tannic

acid inhibits the adsorption of uranine by GAC in solutions with low uranine concentrations. Three of the four experiments resulted in the control sample yielding more uranine than the tannic acid-treated sample (Figure 13). Experiment one showed that the control had 13.51% more than the contaminated sample, experiment two resulted in the control showing 39.25% more than the contaminated sample, and experiment three revealed a control sample that yielded only 0.76% more mass than the contaminated sample. Experiment four contrasted sharply with the first three experiments, with a control sample that yielded 85.81% less than the tannic acid treated sample.

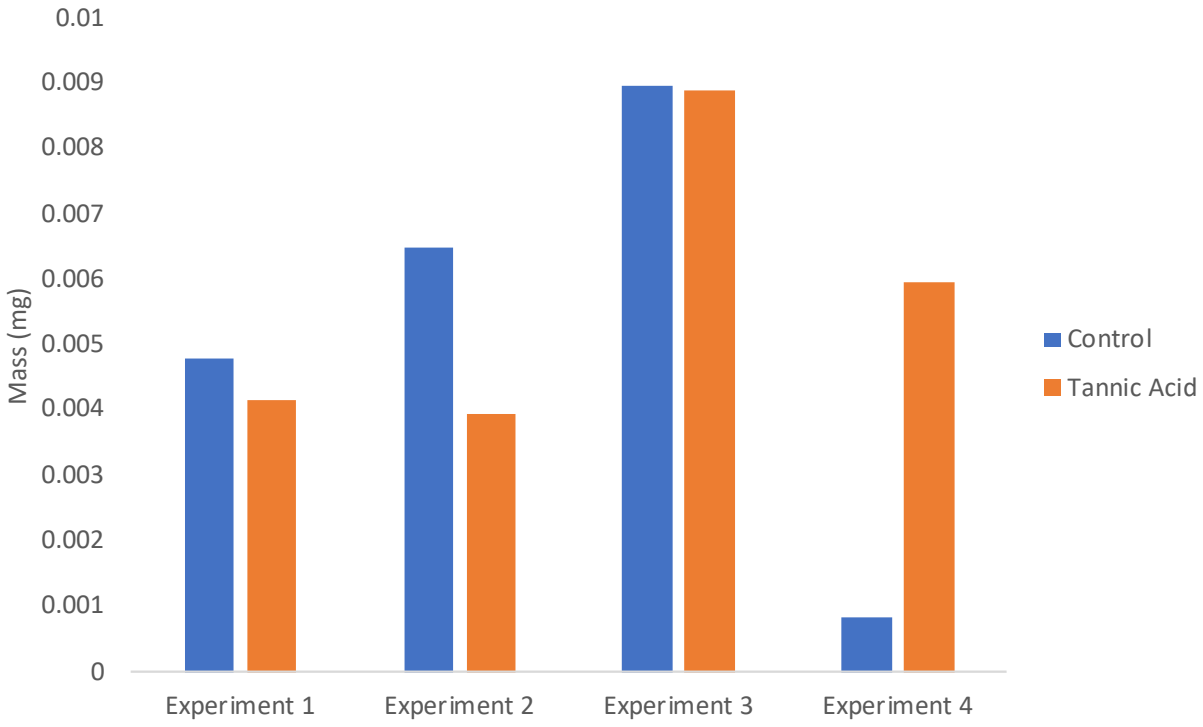


Figure 13. Mass of dye extracted from GAC in four MFR experiments where one sample was injected with tannic acid solution prior to 500 ppb uranine solution (orange) and the other acted as a control using Nanopure (blue).

In the second method, 60 mL of 500 ppb uranine (0.03 mg) was injected prior to injection of 60 mL of 10 ppm tannic acid (or Nanopure water for a control) in order to determine if tannic acid could desorb the dye from the GAC at lower concentrations of dye (Figure 14). Under these

conditions, the contaminated sample yielded 1.51% more dye than the control sample. As with previous experiments, most of the original dye mass was not absorbed by the GAC.

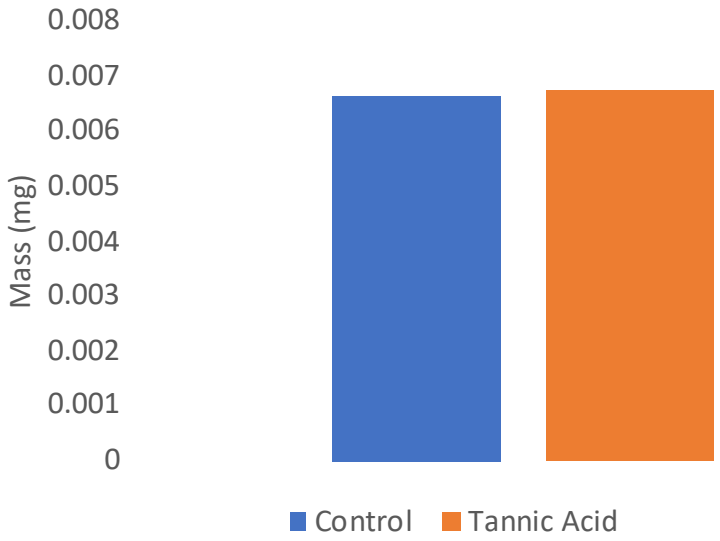


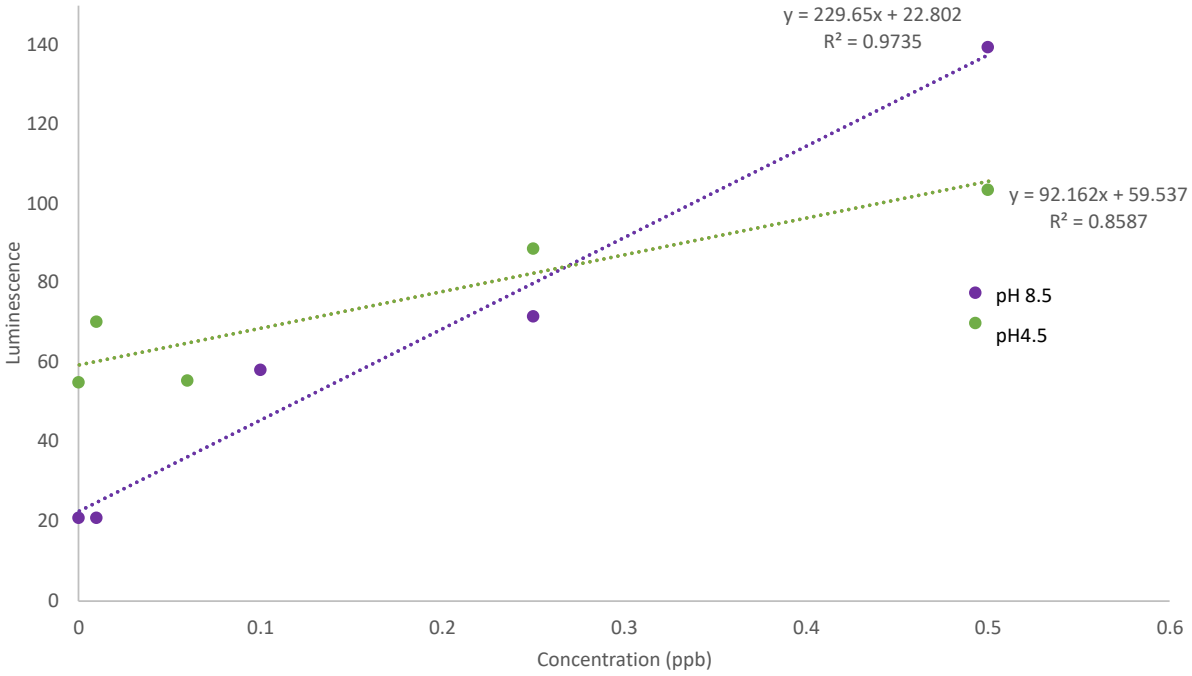
Figure 14. Mass of dye extracted from GAC in two MFR experiments where one sample was injected with 500 ppb uranine solution prior to tannic acid solution (orange) and the other acted as a control using Nanopure (blue)

Effect of pH on dye adsorption

To evaluate the effect of pH on dye adsorption to GAC, two sets of mixed flow reactor experiments were conducted using uranine solutions at concentrations of 0.5, 0.25, 0.1, 0.06, and 0.01 ppb in Nanopure water, then adjusted to either pH 4.5 or 8.5 with 0.5 M HCl or 0.5 M NaOH, respectively. Experiments with an injection solution pH of 4.5 range between luminescence values of 60 and 100 with an increase in luminescence as the injected uranine concentration increased (linear regression slope = 92.16), suggesting more dye was adsorbed to GAC with increasing concentration (Figure 15A). The experiments with an injection solution of pH 8.5 range in luminescence from values of 20 to 140, with an increase in luminescence as the injected uranine concentration increased (linear regression slope = 229.65). Comparing the two datasets, it appears that both experience some background luminescence, even in blank samples.

The larger intercept for the pH 4.5 regression suggests that this background luminescence effect is amplified for experiments conducted at lower pH, though the cause remains unknown. If the background luminescence is subtracted from each dataset (Figure 15B), it becomes apparent that experiments conducted at pH 8.5 yield more uranine than experiments conducted at pH 4.5, suggesting that adsorption is enhanced at higher pH and/or inhibited at lower pH for the range of uranine concentrations used in these experiments.

A



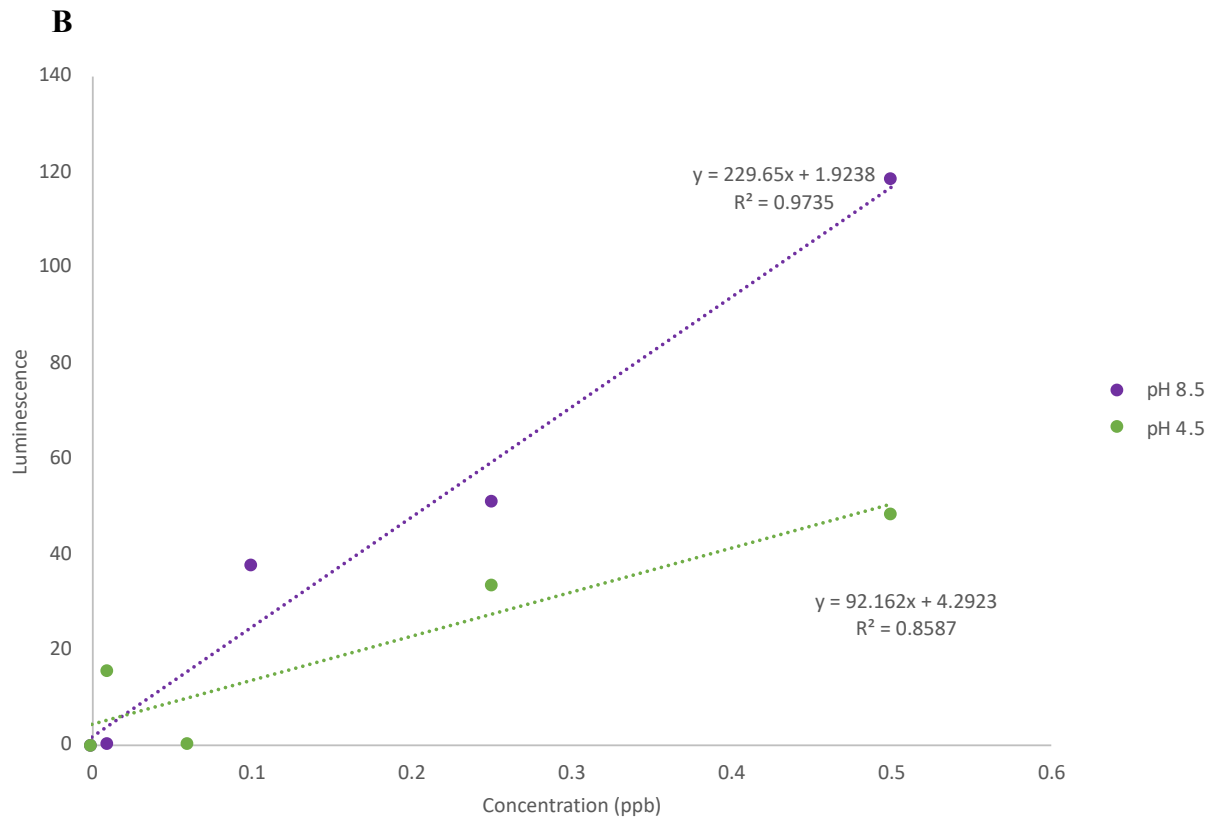


Figure 15. A) Concentration vs luminescence from experiments with initial water solutions adjusted to pH 8.5 (purple) and pH 4.5 (green). Initial concentration of dye in water is plotted on the x-axis and the luminescence response of dye in KOH extracted from GAC is plotted on the y-axis. **B)** Data from plot A) corrected to remove background luminescence so that initial values run through the origin, more clearly showing the effect of pH on adsorption.

DISCUSSION

Determining equilibrium time

In the experiments where 30 ppm uranine solutions were monitored over time (Figure 9), dye adsorbs to GAC exponentially, following the first order rate law. The first order rate coefficient, k, for this reaction is -0.0332 hr^{-1} and can be used with the exponential decay formula, $[A]_t = [A]_0 e^{-kt}$ to quantify the concentration at any time, t, given the initial concentration $[A]_0$. The half-life of this reaction is approximately 20 minutes. Thus, after 120 minutes, 6 half-lives have passed, and the reaction has effectively reached equilibrium. With this information, we can assume that our experiments with less mass of initial dye and the same amount of GAC

could proceed with confidence that the dye and GAC would easily reach equilibrium within the experimental 2.5 hours. However, our experiments were conducted in tightly controlled lab experiments, so on a broader scale, hydrogeologists conducting dye tracer tests in the field should aim to place GAC packets in lower energy water so as to allow the dye time to interact with the GAC. If GAC packets are placed in higher energy water, the dye may pass by the packet too quickly for adsorption to take place; thus, leading the researcher to falsely conclude that dye had not passed through the area.

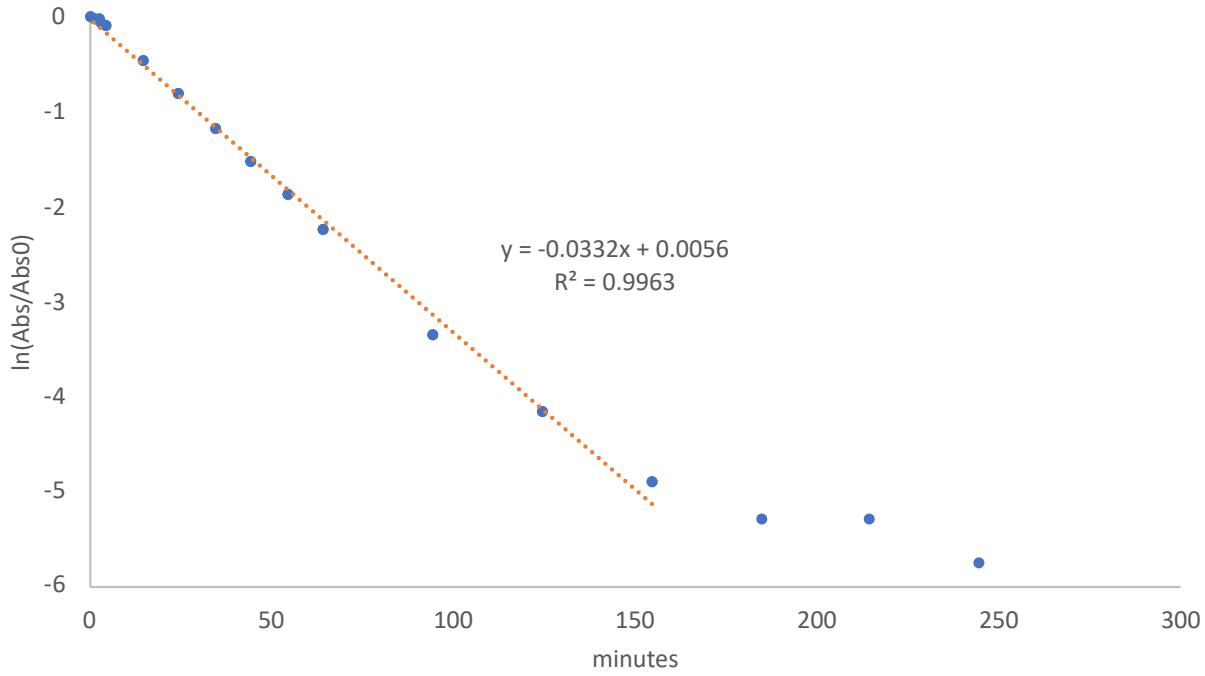


Figure 16. First order rate constant reaction plotted as time vs the natural log of the absorbance at time t relative to the initial absorbance: $\ln(Abs/Abs_0)$. First-order rate coefficient, k = -0.0332 hr^{-1}.

Quantifying dye extractions from batch reactor experiments

The batch reactor experiments, with low initial uranine concentrations (from 2.5- 20 ppb) and dye extracted with KOH, show a general trend of increasing uranine extracted from GAC as the initial uranine concentration increased (Figure 10; R^2= 0.8567). It is important to note that

there was significant variability between replicates of these experiments, as seen by the error (Figure 10). However, the quasi-linear trend between the initial uranine concentration and luminescence is valuable because it provides semi-quantitative constraints for uranine extracted from field GAC packets, which previously was strictly interpreted as a binary result. The regression curve formula allows hydrogeologists to conduct a dye tracer test in the field and to calculate the concentration of dye that was adsorbed by the field packet. With this information, hydrogeologists can begin to constrain properties such as flowpath length and dilution as a result of mixing along the groundwater flowpath in addition to the simple flow direction that is normally gathered from dye tracer testing.

Impact of tannic acid on adsorption of dye to GAC

The varied and inconsistent results from the experiments conducted by injecting tannic acid into the mixed flow reactor prior to 20 ppm uranine dye solutions indicate that tannic acid does not have a strong or consistent effect on the adsorption of 20 ppm uranine (Figures 11 and 12). Experiments that led to more dye in the tannic acid-treated sample than the control indicate that it might be possible for tannic acid to adsorb to GAC, which then serves as a ternary complex and sorbs potentially multiple uranine molecules in the formation of a ternary complex. In effect, this results in more moles of adsorbed uranine per adsorption site, resulting in an increased adsorption capacity for dye. This hypothesis is described in Eqs. 1-3. Eq. 1 serves as a model for the control experiment, where it is clear that one adsorption site on GAC ($R\text{-}CO^-$) can only sorb one uranine ($C_{20}H_{12}O_5$) molecule.

$$R\text{-}CO^- + C_{20}H_{12}O_5 = R\text{-}CO\text{-}C_{20}H_{12}O_5 \qquad \text{(Eq. 1)}$$

In the hypothesized ternary complex model, tannic acid (TA) has *n* number of viable functional groups that can undergo adsorption (ones uninhibited by electrostatic repulsion, steric hindrance etc.). When tannic acid sorbs to GAC (Eq. 2), one adsorption site is lost, leaving *n-1* adsorption sites to potentially sorb *n-1* uranine molecules (Eq. 3). If $n > 1$ given the molecular geometry and appropriate electrostatic conditions, it is possible that tannic acid adsorption onto GAC might increase the overall adsorption of uranine. This hypothesis is presented conceptually in Figure 17. More work needs to be done to confirm those results because experiments in which more dye was yielded from contaminated samples was only observed twice, although the yield differences were not insignificant (14.06% and 46.65%).

$$R\text{-}CO^{-} + [TA]_{n} = R\text{-}CO\text{-}[TA]_{(n-1)} \qquad \text{(Eq. 2)}$$

$$R\text{-}CO\text{-}[TA]_{(n-1)} + m C_{20}H_{12}O_5 = R\text{-}CO\text{-}TA\text{-}[C_{20}H_{12}O_5]_{(n-1)} + (m-(n-1))C_{20}H_{12}O_5 \qquad \text{(Eq. 3)}$$

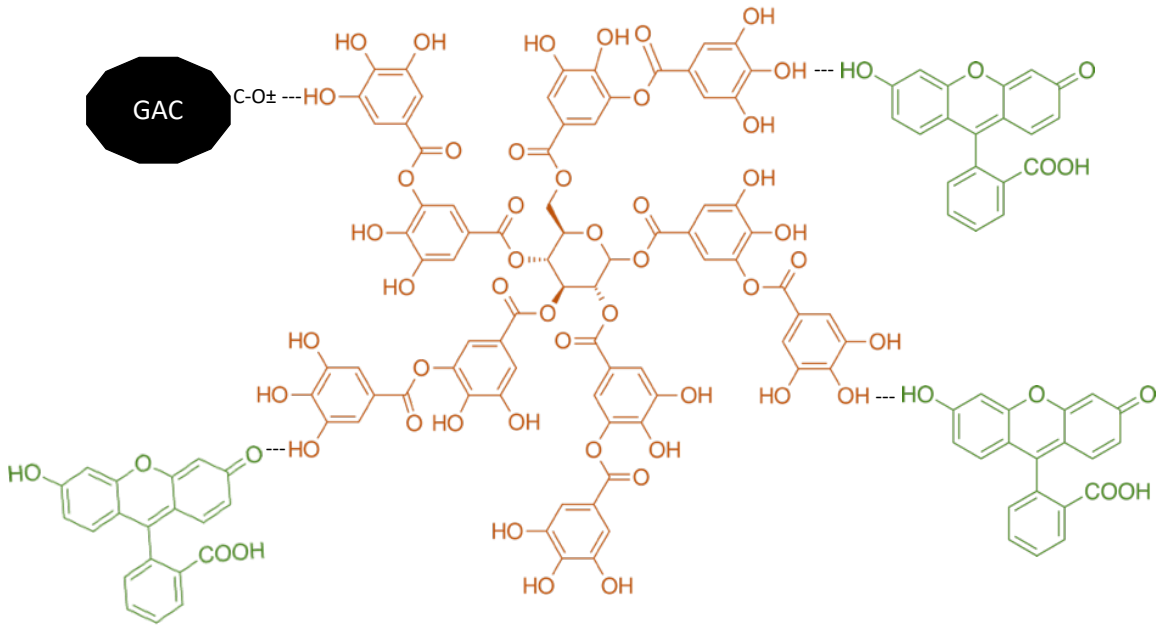


Figure 17. Tannic acid (orange) interaction with GAC to form a complex that adsorbs multiple uranine molecules (green) in the formation of a ternary complex.

The two experiments executed by injecting tannic acid after the uranine dye solution show that tannic acid does not appear to desorb dye from GAC after the dye has already adsorbed. This may be due in part by the large structure of a tannic acid molecule, and it is sterically hindered from approaching the GAC surface by adsorbed uranine molecules.

Experiments conducted by injecting tannic acid solution into the mixed flow reactor before the 500 ppb uranine dye solution showed varied results, similar to the 20 ppm dye solution (Figure 13). In experiments one and two, the control samples yielded more dye than the tannic acid treated samples, indicating that tannic acid may hinder dyes ability to adsorb to GAC. Experiment three resulted in an indistinguishable difference between the control and the tannic acid treated sample. The extreme difference between the control and contaminated sample in Experiment 4 of this injection method indicates that tannic acid may have the ability to adsorb to GAC and aid in the adsorption of uranine dye at low concentrations (Figure 17), but since this

experiment is such an outlier, more experiments will need to be conducted to confirm that conclusion as it was only observed once.

Only one experiment was conducted by injecting 500 ppb uranine dye before the tannic acid solution, and the results were analytically indistinguishable (Figure 14). More research must be done to conclude whether or not tannic acid has the ability to desorb dye at low concentrations from GAC.

Impact of pH on adsorption of dye to GAC

Experiments done with altered pH show that injection solutions adjusted to pH 4.5 had less uranine extracted than experiments in which the injection solutions were adjusted to pH 8.5, suggesting dye was less effective at adsorbing to GAC at pH 4.5. At pH 8.5, uranine exists predominantly as a di-anion and carries two sites of negative charge due to deprotonation of carboxyl and phenolic functional groups (Figure 18). At pH values near 4.5, uranine exists primarily as a mono-anion with only one negative charge site from the deprotonation of the carboxyl group (Figure 18). These lesser charged uranine forms, neutral and mono-anion, have less opportunity to interact with the functional groups on GAC. Thus, when dye is in 4.5 pH and takes a mono-anionic form, it is unable to sorb to GAC in the same capacity as dye in di-anionic form at 8.5 pH. In addition, the pKa range for the transition from the mono-anion to the neutral structures occurs over a range of pH 4-5. Regardless of the exact pKa, this means that an appreciable amount of uranine molecules are in the neutral, uncharged form, further inhibiting the ability to sorb to GAC. This indicates that dye tracer testing done in the field may not be effective in slightly- to-very acidic conditions.

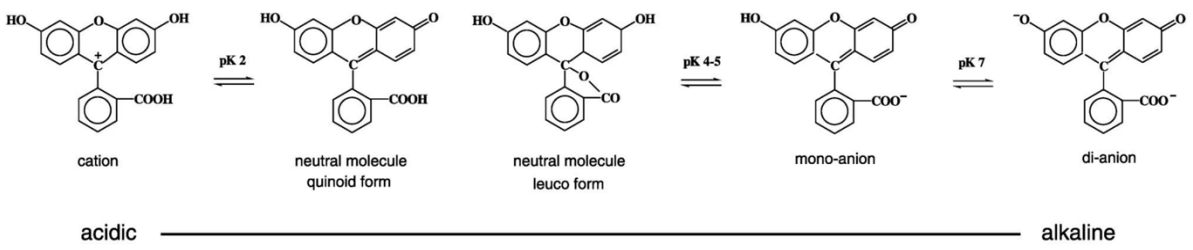


Figure 18. Structural form of uranine dye (adapted from Flury and Wai, 2003) showing changes in form due to changes in pH.

In addition to pH causing protonation/deprotonation reactions on uranine functional groups, pH conditions could also have an effect on the functional groups of GAC. GAC is a complex molecule that does not have a single molecular structure; each molecule differs. The most common structures of GAC include a combination of phenolic and carboxyl functional groups. At pH values near 4.5, both phenolic and carboxyl groups have neutral charge. Whereas in pH conditions near 8.5, phenolic and carboxyl groups are negatively charged. These changes in the charge of the functional groups have an effect on whether uranine is able to electrostatically interact with GAC, perhaps through the formation of hydrogen bonds.

CONCLUSION

Investigating the environmental factors that could impact dye tracer testing in the field helps ensure that the method is executed to its fullest potential by providing hydrogeologists with guidelines for when and where dye tracer testing may be more or less effective. Through the experiments outlined in this paper, three aspects of dye tracer testing have been elucidated: how much dye can adsorb to GAC in a given amount of time, how tannic acid affects adsorption, and how pH affects adsorption. GAC is most effective at adsorbing uranine during the first 30 minutes of interaction in a controlled environment, so scientists working in the field should limit placement of GAC packets to environments with relatively low energy to allow for maximum

adsorption. GAC packets interacting with 20 ppm uranine dye are not susceptible to competitive adsorption with tannic acid, but more research needs to be conducted to determine if tannic acid can desorb dye from GAC. Hydrogeologists should be wary of tannic acid when considering GAC packet placement because tannic acid may have the ability to take up sorption sites and hinder low concentrations of dye from adsorbing to GAC. The pH of dye-water solutions does have an effect on adsorption due to the pH-dependent functional groups of both dye and GAC molecular structures where pH values of 4.5 result in less adsorption to GAC than pH 8.5. Our results suggest that hydrogeologists should avoid conducting dye tracer testing in acidic waters, as water with low pH could lead to false negative results.

These experiments do not address all of the uncertainties associated with dye tracer testing, so more work needs to be done to determine how environmental factors affect this method. For instance, future researchers should investigate how dye and tannic acid interact with GAC when mixed in a single solution. More work also needs to be done to build upon our current findings, especially for experiments conducted only once. Studies like these are crucial because dye tracer testing helps elucidate the complexities of karst aquifers, providing important data to monitor and protect the water resources that support millions of people.

REFERENCES

Flury, M., and Wai, N.N., 2003, Dyes as tracers for vadose zone hydrology: Reviews of Geophysics, v. 41, doi:10.1029/2001RG000109.

Ford, D., and Williams, P., 2013, Karst Hydrogeology and Geomorphology:, doi:10.1002/9781118684986.

Hauwert, N., Johns, D., and Hunt, J., 2004, Flow systems of the Edwards Aquifer Barton Springs Segment interpreted from tracing and associated field studies: Edwards Water …, p. 1–18, http://www.bseacd.org/uploads/Hauwert et al_, 2004, Edwards Symposium.pdf.

Hunt, B.B., Smith, B.A., Campbell, S., Beery, J., Hauwert, N., and Johns, D., 2005, Dye tracing recharge features under high-flow conditions , Onion Creek , Barton Springs Segment of the Edwards aquifer , Hays County , Texas: Austin Geological Society Bulletin, v. 1, p. 70–86.

Kaçaroğlu, F., 1999, Review of groundwater pollution and protection in karst areas: Water, Air, and Soil Pollution, v. 113, p. 337–356, doi:10.1023/A:1005014532330.

Kletetschka, K., Rimstidt, J.D., Long, T.E., and Michel, F.M., 2018, Suitability of 3D-Printed devices for low-temperature geochemical experiments: Applied Geochemistry, v. 98, p. 121–126, doi:10.1016/j.apgeochem.2018.08.012.

Michel, F.M., Rimstidt, J.D., and Kletetschka, K., 2018, 3D printed mixed flow reactor for geochemical rate measurements: Applied Geochemistry, v. 89, p. 86–91, doi:10.1016/J.APGEOCHEM.2017.11.008.

Rivera-Utrilla, J., Moreno-Castllla, C., Utrera-Hldalgo, E., and Carrasco-Marln, F., 1993, Removal of Tannic Acid from Aqueous Solutions by Actvated Carbons: The Chemical Engineering Journal, v. 52, p. 37–39.

Shockey, C.R., 1996, The Enigma of the Blind Salamander and Groundwater Pumping : Lessons

from the Edwards Aquifer , Texas:

Smart, P.L., and Laidlaw, I.M.S., 1977, An evaluation of some fluorescent dyes for water tracing: Water Resources Research, v. 13, p. 15–33, doi:10.1029/WR013i001p00015.

Smart, C., and Simpson, B., 2002, Detection of fluorescent compounds in the environment using granular activated charcoal detectors: Environmental Geology, v. 42, p. 538–545, doi:10.1007/s00254-001-0517-4.

Smith, B.A., Hunt, B.B., and Schindel, G.M., 2005, Groundwater flow in the Edwards Aquifer: Comparison of groundwater modeling and dye trace results: Geotechnical Special Publication, v. 40796, p. 131–141, doi:10.1061/40796(177)15.

Taucer, P.I., Munster, C.L., Wilcox, B.P., Shade, B., Dasgupta, S., Owens, M.K., and Mohanty, B., 2005, Large plot tracing of subsurface flow in the Edwards Aquifer epikarst: Geotechnical Special Publication, v. 40796, p. 207–215, doi:10.1061/40796(177)22.

Weary, D.J., and Doctor, D.H., 2014, Karst in the United States: A Digital Map Compilation and Database: , p. 23, doi:http://dx.doi.org/10.3133/ofr20141156.